ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS

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BY

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Dr. Pulkit Dhiman…

ABBREVIATIONS

IBD –	Inflammatory Bowel Disease
UC –	Ulcerative Colitis
CD –	Crohn's Disease
FMT –	Faecal Microbiota Transplantation
CDI –	Clostridium Difficile Infection
GWAS –	Genome Wide Association Study
TNFSF –	Tumour Necrosis Factor Super Family
TNF-α –	Tumour Necrosis Factor Alpha
TLR –	Toll like Receptor
MDR –	Multidrug Resistance
SNP –	Single Nucleotide Polymorphism
rRNA –	Ribosomal Ribonucleic Acid
IL –	Interleukin
ACCESS –	Asia-Pacific Crohn's and Colitis Epidemiology Study
GI –	Gastrointestinal
PUCAI –	Paediatric Ulcerative Colitis Activity Index
RCT –	Randomised Control Trial
ITT –	Intention to Treat
hs CRP –	high sensitivity C - reactive protein
E –	Extent

PIS –	Patient Information Sheet
DMCH -	Dayanand Medical College and Hospital
E. coli –	Escherichia Coli
ACT –	Active Ulcerative Colitis Trial
ULTRA –	Ulcerative Colitis Long Term Remission and Maintenance with Adalimumab
PURSUIT-SC –	Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment-Subcutaneous
PGA –	Physician Global Assessment
HCV –	Hepatitis C Virus
HbsAg –	Hepatitis B Surface Antigen
HIV –	Human Immunodeficiency Virus
IVDU –	Intravenous Drug Use
BMI –	Body Mass Index
IBS –	Irritable Bowel Syndrome
M: F –	Male: Female
Aza/6MP –	Azathioprine/6 Mercaptopurine
5-ASA –	5-Amino Salicylic Acid
PEG –	Polyethylene Glycol
Std. –	Standard
No. –	Number
%age –	Percentage

KEY TO MASTER CHART

S. No.	Serial Number
IVDU	Intravenous Drug Use
BMI	Body Mass Index
Kg/m^2	Kilogram/Meter Square
Aza/6MP	Azathioprine/6 Mercaptopurine
5-ASA	5-Amino Salicylic Acid
hs CRP	high sensitivity C - reactive protein
E	Extent
N	No
Y	Yes
NA	Not Available
M	Male
F	Female

ABSTRACT

Introduction: Faecal microbiota transplantation (FMT) as a potential treatment for inflammatory bowel disease (IBD) is an area of active current research, taking into account of its success in the treatment of Clostridium difficile-associated colitis. Faecal microbiota transplantation (FMT) is the artificial replacement of a 'dysbiotic' microbiota with one from a healthy individual.

Aim: To study the role of fecal microbiota transplantation in patients with steroid refractory Ulcerative Colitis.

Methods: This is an interventional, prospective and uncontrolled study. Patients who have moderately active disease based on Mayo Clinic score of 6 to 10, refractory to steroids are included. FMTs are done at week 0, 2, 6, 10, 14, 18 and 22 weeks. Primary outcome (Remission) and Secondary outcome (Clinical response and Mucosal healing) is calculated at week 22 (seventh FMT). Data were described in terms of range; mean ± standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate.

Results: Sixteen patients were found to have steroid refractory UC during the study period (1st March 2018 till 31st August 2019). Out of sixteen patients, two patients were lost to follow up, one patient did not tolerate FMT (Pain Abdomen) and one patient died due to severe disease during the study period. Twelve patients with moderately active Ulcerative Colitis (Mayo Clinic score - 6 to 10) were included in the final analysis. These patients received FMT, clinical response and disease activity was assessed by full Mayo Clinic Score. Disease activity improved with each session (mean Full Mayo score 7.66 ± 1.15 at baseline vs 3.3 ± 2.34 at session 7). Primary outcome was achieved in six (50%) out of twelve patients. Clinical response and mucosal healing (secondary outcome) was achieved in eight (66.6%) patients and seven (58.33%) patients respectively.

Conclusion: Intensive-dosing, multidonor, faecal microbiota transplantation induces clinical remission and endoscopic improvement in patients with steroid refractory active ulcerative colitis. The procedure was relatively safe with no serious adverse events. So, FMT could be considered as a potential rescue treatment modality in patients with refractory UC.

Keywords: Faecal Microbiota Transplantation, Ulcerative Colitis, Dysbiosis, Mayo Clinic score.

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INTRODUCTION

Inflammatory bowel disease (IBD) largely comprises Crohn's disease and ulcerative colitis which runs a relapsing, remitting course and tends to occur in the second or third decade of life.[1] The prevalence of IBD is greatest in the developed world affecting one million people in USA, 2.5 million in Europe and the prevalence is increasing in developing countries. Recent studies from China, India and Middle East reported prevalence of between 2.0 and 6.0 per 100,000 individuals.[2]

Inflammatory Bowel Disease (IBD) is the result of a chronic inflammatory process deriving from disequilibrium between self-microbiota composition and immune response. The etiology of IBD is still unknown, but several factors have been identified for induction and alteration of mucosal immune system and loss of immune tolerance, associated with dysbiosis (altered balance of beneficial and aggressive bacteria in gut).[3]

The human gastro-intestinal tract is home to about 1000 different species of bacteria along with viruses and yeasts which represents 100 fold greater genetic material than found in human body. It is involved in short-chain fatty acid metabolism, prevention of invasion by pathogens and preservation of gut epithelial barrier. Colonic microbiota is largely composed of members of two predominant phyla, gram positive Firmicutes (about 35%) and gram negative Bacteroides (about 60%). The remaining is representative of gram negative Proteobacteria, Actinobacteria and Eucharyotes.

Patients with IBD generally have lower bacterial diversity. Microbial segregation was found as compared to healthy individuals in terms of decreased Firmicutes and increase in population of Proteobacteria.[4]

Faecal microbiota transplantation (FMT) is the artificial replacement of a 'dysbiotic' microbiota with one from a healthy individual. Faecal microbiota transplantation entails transfer of the entire enteric microbiota and might have augmented capacity to correct the complex microbial disturbances associated with ulcerative colitis.[5] Fecal transplantation has attracted immense interest in recent years and has

been shown to be effective for relapsing Clostridium difficile infections. Current medical treatment of IBD remains imperfect and a significant number of patients need colectomy to manage their disease.

With advances in understanding of the gastrointestinal microbiota in terms of structure and function, FMT seems an exciting alternative for management of UC. Achieving and sustaining clinical remission on a long term basis have been the primary goal of treatment.

In recent years, many studies are coming up which report beneficial effect of FMT in induction and maintenance of remission in patients with active Ulcerative Colitis. This study intends to look into the role of FMT in patients with steroid refractory Ulcerative Colitis.

AIMS AND OBJECTIVES

- To study the role of Fecal microbiota transplantation in patients with steroid refractory Ulcerative Colitis.

REVIEW OF LITERATURE

Ulcerative colitis (UC) is a relapsing remitting disorder characterized by inflammation limited to mucosal surface of the large bowel. The disease starts in the rectum and in most cases extends proximally in a continuous fashion. UC is classified by the extent of colonic involvement, from proctitis to left-sided colitis or extensive colitis (pancolitis).[6]

Epidemiology: Western world bears the maximum brunt of disease with incidence rates of 6–15.6 and 10–20.3 per 100,000 in North America and North Europe, respectively.[7] Inflammatory bowel disease (IBD), once considered to be a Western disease, is increasingly being reported from India and other Asian countries. The study of IBD epidemiology in these regions has been hampered by a lack of universal accessibility to health care, hospital-based studies, the absence of standardized case definitions and relevant databases.

The Indian disease pattern is going through a phase of epidemiological transition with a surge in the incidence of IBD. There are two studies, both from northern states, which have reported the population prevalence of UC. In a house to house survey of 21,971 persons in Haryana in 1984, a prevalence rate of 42.8 per 100,000 population was recorded.[8] The second study, conducted in Ludhiana, Punjab, screened a population of 51,910 through a cluster random sampling method, and prevalence rate of 44.3 per 100,000 population was documented. The crude incidence rate calculated after a second visit 1 year later was 6.02 per 100,000 population.[9] The data indicated that UC is not rare in India.

North-South Gradient:

IBD burden is much higher in the North as compared to the South as observed in studies from the USA and Europe.[10] Different exposure to sunlight and hence to vitamin D may account for such a North-South gradient in the West.[11] In a survey published from India in 2012, it revealed that IBD was seen in all parts of North and South India.[12]

India being a tropical country may not follow this North- South gradient in terms of sunlight exposure. The overall estimated IBD population in India in 2010 came out to be 1.4 million, which was the second highest number after the USA (1.64 million).[13]

Genetics: Recent genome wide association searches have uncovered over 150 potential genes associated with IBD.[14] The initial data for the GWAS originated primarily from Europe, since then, many such publications have come up from Asia, including Japan, Korea, and India. These studies demonstrate both similarities and dis-similarities in the genetic architecture of Caucasians and non-Caucasians. One of the studies found a weak association ofrs2066842 (Pro268Ser) with UC.[15] An Indian study reported a strong protective association with TNFSF15 (tumour necrosis factor superfamily) gene polymorphisms and IBD in the Indian population.[16]

Another study found that the TLR5 variants R392Xand N592S showed significant association with UC.[17] The study also concluded that patients having a combination of multiple SNPs, both in the TLR5 and the TLR4 gene suffered from early-onset disease and had a severe long-term course. MDR1 (ABC B1) gene polymorphisms were associated with early age at disease onset, left-sided disease, and steroid response in UC.[18] A trans-ethnic study demonstrated a shared genetic risk across different populations along with genetic heterogeneity with NOD2 polymorphisms being absent in Asians and TNFSF15 having a much larger effect size in Asians as compared to Caucasians.[19]

Microbiome: It has long been thought that gut microbiota is likely to be the key to the pathogenesis of IBD. Gut microbiome studies in IBD patients have demonstrated a reduced diversity in the bacterial population, especially within the Firmicutes phylum. A study analysed mucosa associated bacterial flora from control individuals and IBD patients by real-time analysis using 16S rRNA-based genus-specific primers.[20] They found that There was a significant decline in the population of Bacteroides, Lactobacillus, Ruminococcus and Bifidobacterium bacteria in both UC and CD patients.[21] In contrast to

the mucosal studies, the study found that Lactobacilli in the faecal samples of patients with active UC are significantly increased as compared to healthy controls. These levels of Lactobacilli significantly reverted back to normal during remission, and reached levels close to those of controls. The findings replicated results from other studies from the West.[22]

Microbiome signature in studies from Indian IBD population matches that of patients in the West, indicating that the players in the pathogenesis of IBD are not different between the West and the East.

Environmental Factors: The Hygiene Hypothesis: Literature supporting the hygiene hypothesis is inconsistent both in India.[23,24] as well as other Asian countries [25] as shown in table 1

Table 1: Environmental risk factors

Factor	India		ACCESS	
	UC ($n = 518$)	CD ($n = 200$)	UC ($n = 186$)	CD ($n = 256$)
Breast feeding >12 months	–	–	protective	protective
Childhood pets	prone	no effect	protective	protective
Antibiotic use	–	–	protective	protective
In-house water tap	no effect	prone	protective	no effect
Bed sharing	prone	–	–	–
Toilet facilities	protective	no effect	protective	no effect
Higher number of siblings	no effect	–	–	–
Cattle in the house compound (recently)	–	protective	–	–

In a country-wide survey of IBD from India, the mean age at diagnosis of UC and CD was 38.5 and 35.9 years, respectively, and was not associated with a second peak as observed in the data from the West. Gender distribution in UC is either equal to or associated with a sight male preponderance, whereas in CD it is consistently associated with a female preponderance in the west. The IBD survey from India reported a male: female ratio of 1.4 and 1.3 for UC and CD, respectively. The male preponderance could be attributed to socio-referral bias, where women and girls are underrepresented in receiving medical attention.[26]

Pathophysiology

Pathophysiology of UC is complex. Epithelial barrier is the first line of defence which is lost in UC as disruption of tight junctions and the mucus film covering the epithelial layer leads to increased uptake of luminal antigens (commensal microbiota). Macrophages and dendritic cells recognise the non-pathogenic bacteria through molecular pattern recognition receptors (TLR) and their functional status changes from tolerogenic to an activated phenotype. This results in increased production of proinflammatory cytokines (TNF-α, interleukins 12, 23, 6, and 1β).[27] Various bacteria have been shown to differentially activate helper T cells, such as pro-inflammatory Th-17 cells or anti-inflammatory regulatory T cells. Some have also been found to secrete metabolites that reduce the production of the pro-inflammatory cytokines IL-2 and interferon-gamma and increase levels of anti-inflammatory cytokines such as IL-10.[28] High levels of intestine specific IL-10 are thought to mediate the communication between the systemic immune response and the gut epithelium, resulting in a decrease of pro-inflammatory cytokine release.[29]

Mucosal immune system has two important functions. It needs to be tolerant of the overlying microbiota to prevent the induction of an excessive and detrimental systemic immune response and it needs to be able to control the gut microbiota to prevent its overgrowth and translocation to systemic sites. Gut microbiota is hence intricately involved in determining the efficacy of mucosal immune response. Tolerance at the GI mucosa is interplay of three moieties: modification of the antigenic moieties of the microbiota, modulation of the host response and physical separation of the bacteria and the host cells. Gut microbiota provides physical barrier to incoming pathogens by competitive exclusion, occupation of attachment sites, consumption of nutrient sources and production of antimicrobial substances.

The human gastrointestinal tract is in a persistent state of delicate balance between the non-pathogenic commensals and the enteric pathogenic bacteria. Any imbalance in this intricate state of homeostasis will lead to chaos in the form of changes in the

inflammatory response, altered nutrient milieu, reduction in indigenous anaerobes, competitive advantage to pathogens and resultant instability in the indigenous microbial population which can result in impaired mucosal healing, chronic mucosal inflammation and colitis.

Fecal Microbiota Modulation

Interest is growing worldwide for FMT as a 'natural' therapy from both patients' and physicians' perspective.

Early investigators considered Crohn's disease to be variant of Johne's disease, which is caused by Mycobacterium paratuberculosis and affects cattle.[30] It has been previously shown that infection with Campylobacter and non-typhoid Salmonella increases the risk of developing IBD.[31]

FMT have been used since fourth century when two Chinese physicians Ge Hong and Li Shizen first described the use of fresh or fermented faecal products, called 'yellow soup', to treat severe diarrhoea, fever, pain and constipation.[32]

FMT for the treatment of patients with pseudomembranous colitis was first reported in 1958.[33] In hindsight, these patients were in fact colonized by an overgrowth of toxigenic Clostridium difficile as is commonly seen in elderly patients treated with broad spectrum antibiotics. First report of the use of FMT to treat CDI came in 1983. The first randomized controlled trial for FMT in this setting was reported in 2013. Encouraged by the success of FMT in C. difficile, various trials have come up regarding the role of FMT in IBD.

In 1989, Justin Bennet and Mark Brinkman first reported sustained remission when Dr Bennet treated his own refractory UC with enema delivered stool from a disease free donor.[34]

In 2012 Angelberger et al. reported their results of FMT used to treat five patients with immunotherapy-refractory UC. Only one patient responded following 3 days treatment using FMT by a combined nasojejunal plus enema-delivered approach and this patient's microbiota resembled that of the donor.[35]

A paediatric study with 10 patients aged between 7 and 21 evaluated the efficacy of freshly prepared faecal enemas for 5 days in treating

mild to moderate UC. In this study, seven of the nine subjects showed clinical response within 1 week, six of the nine subjects maintained clinical response at 1 month with a significant reduction in the paediatric UC activity index.[36]

Moayyedi et al. reported the results of a double blinded randomized placebo controlled trial using FMT in the form of an enema administered weekly over 7 weeks to patients with active UC. In this study, clinical remission is seen in 9 out of 38 (24%) patients in the active arm compared to 2 out of 37 (5%) in the placebo group. Eight of the nine patients in remission remained in remission after one year. Patients who were in remission had an increase in microbial diversity and there were no differences in serious adverse events between the FMT and placebo. Investigators noticed that patients diagnosed within a year of treatment were more likely to respond.[37]

Rossen et al. undertook a double blinded RCT using the naso-jejunal route to deliver FMT to patients with mild to moderately active UC. They chose to use patient's own stool as placebo. At 8 weeks, they found remission in 6 of 23 (26%) donor-FMT treated vs. 8 of 25 (32%) autologous FMT treated patients which showed autologous FMT may not be entirely without biological activity.[38]

In a recent 3-centre study from Australia, the investigators suggested that treatment of UC is likely to require an intensive treatment course. They used multiple donors (3–7 per treatment) in their intensive dosing placebo controlled RCT in patients with mild-moderate ulcerative colitis. Patients on steroids at entry had to undergo a mandatory taper over 8 weeks. The trial achieved its primary endpoint with 11 out of 41 (27%) patients in the active treatment arm compared to 3 out of 40 (8%) achieving steroid-free remission at Week 8 (P = 0.021) but endoscopic remission did not differ between the groups.[39]

In another study from Australia, investigators took a novel approach to FMT preparation. Donor and autologous stool were prepared in entirely anaerobic conditions. They showed that 12/38 (32%) patients achieved steroid-free remission in the active arm compared to 3/35 (9%) patients who received autologous FMT (P = 0.02) in the intention to treat (ITT)

analysis. Similarly clinical response and clinical remission rates were 55% vs. 20% (P < 0.01) and 50% vs. 17% (P < 0.01), respectively.[40]

In a study from Turkey, researchers studied the efficacy of FMT by assessing the clinical and endoscopic response in patients with ulcerative colitis (UC) who had failed anti-inflammatory and immunosuppressive therapy. They reported that twenty one (70%) of the thirty patients showed clinical response, and thirteen (43.3%) of the thirty patients achieved clinical and endoscopic remission at week 12. Nine patients (30%) were accepted as a non-responder at the end of week 12. No adverse events were observed in the majority of patients during FMT and 12 weeks follow-up.[41]

The variability in methodology and conflicting results in the published studies highlight the need for further studies that expand on optimal indications, delivery route, dosage, and frequency.

The first reported FMT in humans was via enema. Since then many cases of FMT via colonoscopy have been reported.[42-47] Another mechanism of FMT available today is the upper GI tract. Historically, fecal transplantation delivery procedures have varied in dispersal location of donated microbiota, volume limits, and method of mixture suspension.

Most of the reports have used fresh donations, which can be defined as unfrozen stool used within 24 h, but preferably within 6 hours. Colonoscopic FMT with frozen standardized donor samples has also been successfully implemented. Meta-analyses have showed that frozen FMT is as effective as fresh FMT.[48]

There are advantages and disadvantages for each modality of administration of FMT, but there is no clear consensus on which implementation methodology offers the greatest benefit.

As the Gut microbiota is not static and the composition is expected to change over a period of time with external influences, so single session of FMT will not cause a sustained change in the intestinal flora and repeated sessions at regular intervals are required for prolonged benefits.

Role of active ingredient in FMT still needs to be defined.[49,50] It is also unknown which particular patient population might benefit most from microbial manipulation.

FMT has the potential for transmitting occult infections even when strict donor screening is performed. The clinician has to estimate the risk whether the donor had recently contracted a transmissible disease, as well as rule out potential exposure to other pathogenic agents that are not identified by laboratory methods. This can be facilitated by eliminating donors with a history of engaging in high-risk behaviors, such as illicit drug use, sexual encounters with multiple partners, unprotected sexual activity, tattoo or body piercing in the past 6 months, current or known exposure to a communicable disease, use of immunosuppressant agents, or antibiotics within the last 3 months.

MATERIALS AND METHODS

STUDY DESIGN

This interventional, prospective and uncontrolled study was conducted on the patients attending the outpatient department or admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India.

Patients coming to outpatient department or admitted indoor shall be offered to undergo FMT. Patients providing informed consent were enrolled for the study. Demographics, drug history, comorbidities, disease characteristics like duration, severity (mild, moderate or severe), extent (Montreal classification; Table 2) and inflammatory markers (hs CRP, when available) were recorded in all patients.

Table 2. Montreal Classification of Extent of UC

Extent	Anatomy
E1 Ulcerative Proctitis	Involvement limited to the rectum (that is, proximal extent of inflammation is distal to the rectosigmoid junction)
E2 Left sided UC	Involvement limited to a proportion of the colorectum distal to the splenic flexure
E3 Pancolitis	Involvement extends proximal to the splenic flexure

Patients were educated about fecal microbiota transplantation (FMT) through a Patient Information Sheet (PIS) which was provided to each patient. Donor faeces (200-300 gm) was diluted in 200-300 ml normal saline making slurry. It was filtered and particulate matter was removed. The resultant solution was administered to the patient through colonoscopy within 6 hours of preparation.

Patients were encouraged to retain the solution for as long as possible. These FMTs were done at week 0, 2, 6, 10, 14, 18 and 22 weeks. Steroids were tapered (5 mg/week) after the enrolment of patients in FMT protocol. Adverse events like pain abdomen, fever, nausea,

vomiting, worsening of diarrhea, abdominal distension, loss of appetite etc will be recorded at every visit.

SOURCE OF DATA

Patients suffering from steroid refractory Ulcerative Colitis and attending the outpatient or admitted indoors in Department of Gastroenterology, DMC & H were enrolled for the study.

METHODS OF COLLECTION OF DATA

Confirmed cases of Ulcerative Colitis who were refractory to steroids were enrolled for the study and FMT were done at week 0, 2, 6, 10, 14, 18 and 22 weeks through colonoscopy/retention enemas.

INCLUSION CRITERIA FOR PATIENTS

- Age ≥18 years.
- Patients who have moderately active disease based on Mayo Clinic score of 6 to 10 and are refractory to steroids [defined as ulcerative colitis that lack a meaningful clinical response (as per Mayo score) to glucocorticoids up to doses of prednisone 40 to 60 mg/day or equivalent within 30 days for oral therapy or 7-10 days for IV therapy]
- Able to give informed consent and/or assent as appropriate.

EXCLUSION CRITERIA FOR PATIENTS

- Patients with severe disease (defined as Mayo Score of >10)
- Patients having toxic megacolon
- Pregnant and lactating ladies
- Prior colectomy
- Positive stool test for any of the following: Clostridium difficile, Salmonella, Shigella, Yersinia, Campylobacter, E. coli by standard stool culture.
- Regular probiotic and antibiotic use within prior 4 weeks to enrollment
- Severe immunodeficiency, inherited or acquired (e.g. HIV, chemotherapy, or radiation therapy).
- Patients unwilling or unable to give consent or participate in all study requirements.

The Mayo Clinic Score (Table 3) is a combined endoscopic and clinical scale used to assess the severity of UC. The Mayo Score is a composite of subscores from four categories, including stool frequency, rectal bleeding, findings of flexible proctosigmoidoscopy or colonoscopy, and physician's global assessment, with a total score ranging from 0–12. Within the endoscopic component of the Mayo Score, a score of 0 is given for normal mucosa or inactive UC, while a score of 1 is given for mild disease with evidence of mild friability, reduced vascular pattern, and mucosal erythema. A score of 2 is indicative of moderate disease with friability, erosions, complete loss of vascular pattern, and significant erythema, and a score of 3 indicates ulceration and spontaneous bleeding. Mucosal healing has been defined as a Mayo endoscopic subscore of 0 or 1 in major trials of biological therapies in UC including ACT-1 and ACT-2, ULTRA-1, ULTRA-2, and PURSUIT-SC. Mucosal healing has been associated with a reduced rate of colectomy, lower rate of relapse of disease and decreased risk for colon cancer in UC patients.

Table 3: Mayo Clinic Score for Ulcerative Colitis

Stool frequency	0 = Normal number of stools for this patient
	1 = 1–2 stools more than normal
	2 = 3–4 stools more than normal
	3 = 5 or more stools more than normal
Rectal bleeding	0 = No blood seen
	1 = Streaks of blood with stool less than one-half of the time
	2 = Obvious blood with stool most of the time
	3 = Blood alone passes
Findings on endoscopy	0 = Normal or inactive disease
	1 = Mild disease (erythema, decreased vascular pattern, and mild friability)
	2 = Moderate disease (marked erythema, lack of vascular pattern, friability, and erosions)
	3 = Severe disease (spontaneous bleeding and ulcerations)
Physician's global assessment	0 = Normal
	1 = Mild disease
	2 = Moderate disease
	3 = Severe disease

INCLUSION CRITERIA FOR DONOR

- Age >18 years.
- Score of 2,3 or 4 on Bristol stool scale. (Fig 1)
- Stool culture and microscopy tests negative for common detectable enteric pathogens (Salmonella, Shigella, Campylobacter, Vibrio parahemolyticus, Vibrio cholera, Candida albicans, E. coli, Clostridium difficile, Entamoebahistolytica, Giardia lamblia, Cryptosporidium, Ascarislumbricoides, Trematodes/tape worms).
- Negative screening for hepatitis A, anti HCV, HBsAg, syphilis and anti HIV within three months of donation.

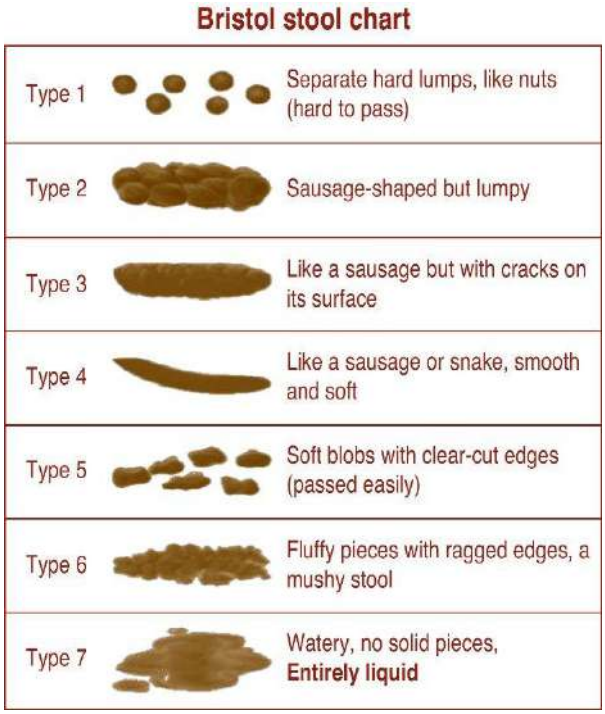
Bristol stool chart
Type 1
Separate hard lumps, like nuts (hard to pass)
Type 2
Sausage-shaped but lumpy
Type 3
Like a sausage but with cracks on its surface
Type 4
Like a sausage or snake, smooth and soft
Type 5
Soft blobs with clear-cut edges (passed easily)
Type 6
Fluffy pieces with ragged edges, a mushy stool
Type 7
Watery, no solid pieces, Entirely liquid

Figure 1: Bristol stool chart

EXCLUSION CRITERIA FOR DONOR

- Tattoos or body piercings within the last 6 months.
- High-risk sexual behaviors.
- Communicable illnesses or antibiotic treatment within the past 3 months.
- Metabolic syndrome, obesity (BMI of >30), or moderate-to-severe undernutrition (malnutrition).

- Intrinsic gastrointestinal illnesses such as irritable bowel syndrome, inflammatory bowel disease, gastrointestinal malignancies or major gastrointestinal surgical procedures.
- A history of malignant illnesses or ongoing oncologic therapy.
- Ongoing immune-modulator therapy for any concurrent illness.
- A history of chronic pain syndromes (fibromyalgia, chronic fatigue) or neurologic, neurodevelopmental disorders.

PROTOCOL FOR PERFORMING FMT

Donor preparation

- A gentle osmotic laxative will be given the night before procedure.
- Donor will be required to avoid any food to which the recipient may be allergic for 5 days before the procedure.
- Donor will be instructed to notify if any symptoms of infection occur between screening and time of donation.

Recipient preparation

- One day prior to FMT, the recipients were advised to take only liquid diet. A night before the procedure, PEGLEC powder dissolved in water was administered to all the patients. Patients were required to pass clear watery stools before they were taken up for the procedure. After the procedure, the patients were encouraged to retain the preparation for as long as possible.

PREPARATION OF STOOL AND ADMINISTRATION

- Stool sample will be collected in an airtight container and will be used within 6 hours of collection.
- Universal precautions will be observed while handling the stool.
- Preservative free normal saline will be used to dilute the stool sample.
- Using a blender, stool will be homogenized and diluents added till it reaches a liquid consistency.
- Stool will be filtered to remove the particulate matter.
- The finished stool slurry will be administered via colonoscope.

DURATION OF STUDY

The study period will start from 1st March 2018 till 31st August 2019

STATISTICAL ANALYSIS:

Data were described in terms of range; mean ±standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations were done using SPSS 21 (Statistical Package for the Social Science) version statistical program for Microsoft Windows.

OUTCOME:

Primary outcome

- Remission (defined as Mayo score of ≤2 with all individual categories ≤1)

Secondary outcome

- Clinical response (defined as decrease of at least 3 points and at least 30% at week 22 versus baseline)
- Mucosal healing (defined as Mayo endoscopy subscore ≤1)

OBSERVATIONS AND RESULTS

This interventional, prospective and uncontrolled study was conducted on the patients attending the outpatient department or admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India. Sixteen patients were found to have steroid refractory UC during the study period (1st March 2018 till 31st August 2019). Out of sixteen patients, two patients were lost to follow up, one patient did not tolerate FMT (Pain Abdomen) and one patient died due to severe disease during the study period. Twelve patients with moderately active Ulcerative Colitis (Mayo Clinic score - 6 to 10) were included in the final analysis. These patients received FMT through per rectal route with colonoscope reaching up to ileum or as close as possible to caecum. Patients were followed-up for their clinical response and disease activity was assessed by full Mayo Clinic Score.

Table 4: Gender Distribution

Sex	No. of cases	Percentage
F	7	58.3%
M	5	41.7%
Total	12	100.0%

The study comprised of 7 (58.3%) females and 5 (41.7%) males, with a M:F ratio of 0.7

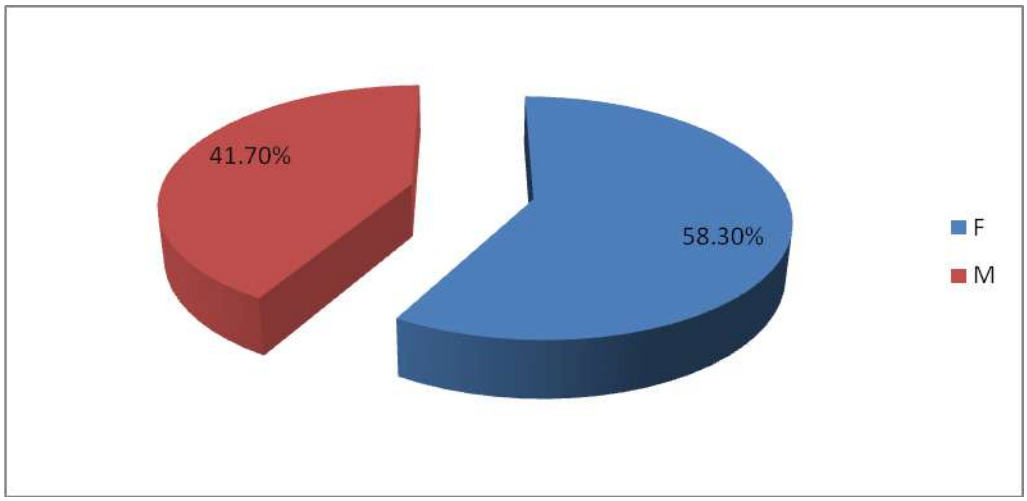

Figure 2: Gender Distribution

Table 5: Age Distribution

	N	Minimum	Maximum	Mean	Std. Deviation
Age	12	18.0	47.0	33.7	9.7

The mean age of study population was 33.7 ± 9.74 (range 18-47) years

Table 6A: Duration of disease

	N	Minimum	Maximum	Mean	Std. Deviation
Duration of Disease	12	1.00	15.00	5.75	5.25

The duration of disease was variable, ranging from 1 year to 15 years. Median time since onset of disease was 3 yrs. Mean disease duration was 5.75± 5.25yrs.

Table 6B: Duration of disease

Duration of diseases	No. of cases	Percentage
<5 years	6	50.0%
6-10.0 years	1	8.3%
> 10 years	5	41.7%

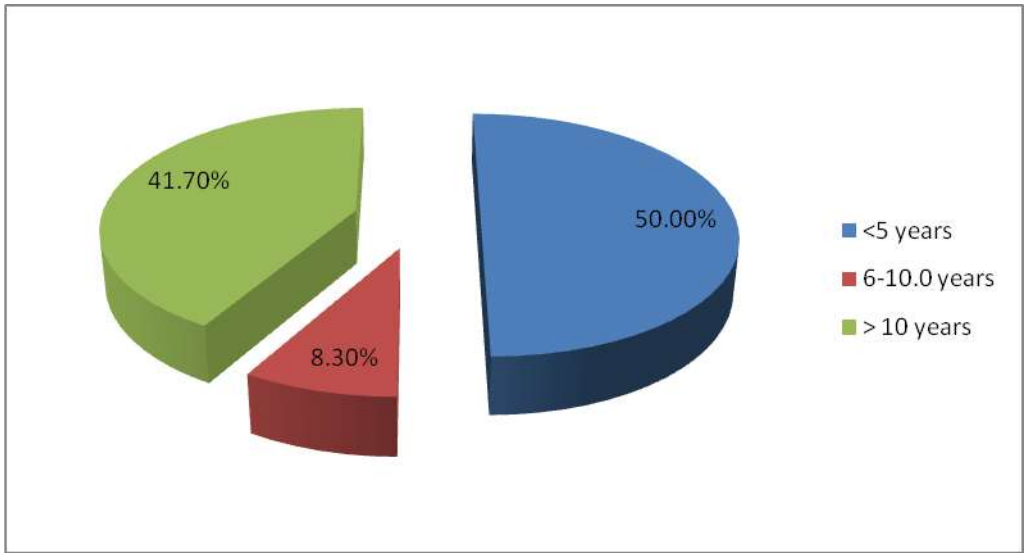

Figure 3: Duration of disease

Table 7A: BMI (kg/m2)

	N	Minimum	Maximum	Mean	Std. Deviation
BMI (kg/m2)	12	16.1	29.8	22.4	3.1

Mean body mass index of subjects was 22.4 ± 3.1 kg/$m^2$with one of the patients being overweight (BMI – 29.8 kg/m^2) and another patient was underweight (BMI – 16.13 kg/m^2)

Table 7B: BMI

BMI	No. of cases	Percentage
Underweight	1	8.3%
Normal	10	83.3%
Overweight	1	8.3%

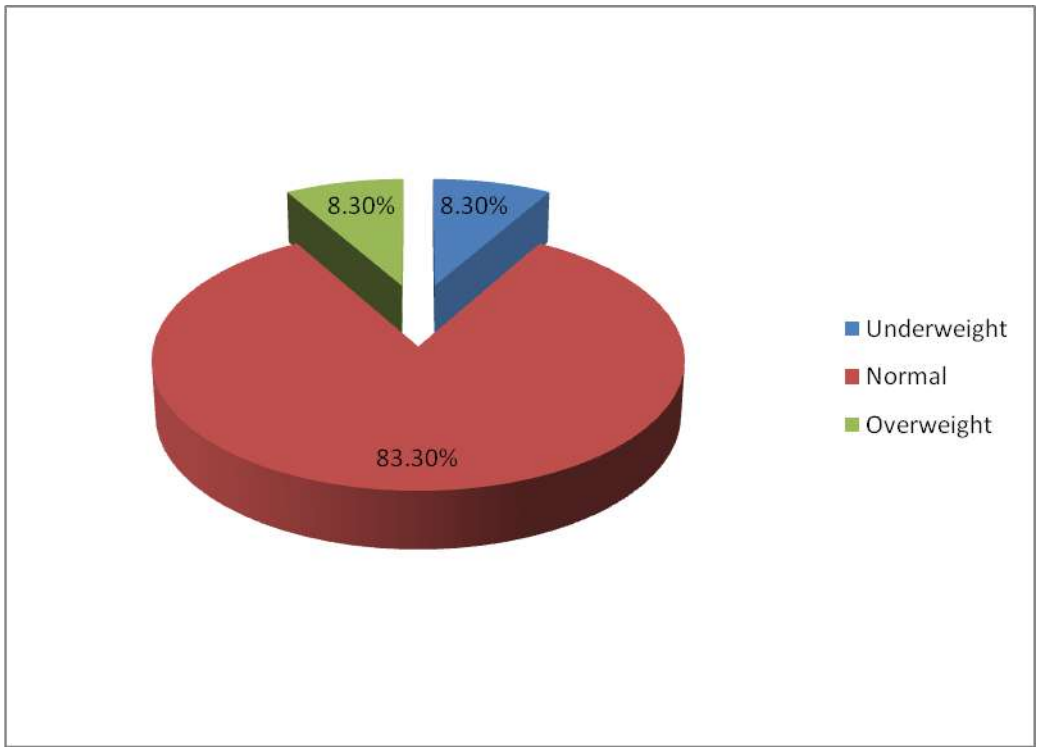

Figure 4: BMI

Table 8: Co-morbidity

Co-morbidity	No. of cases	Percentage
Hypothyroid	3	25.0%
N	9	75.0%
Total	12	100.0%

Three (25%) patients out of twelve had history of Hypothyroidism.

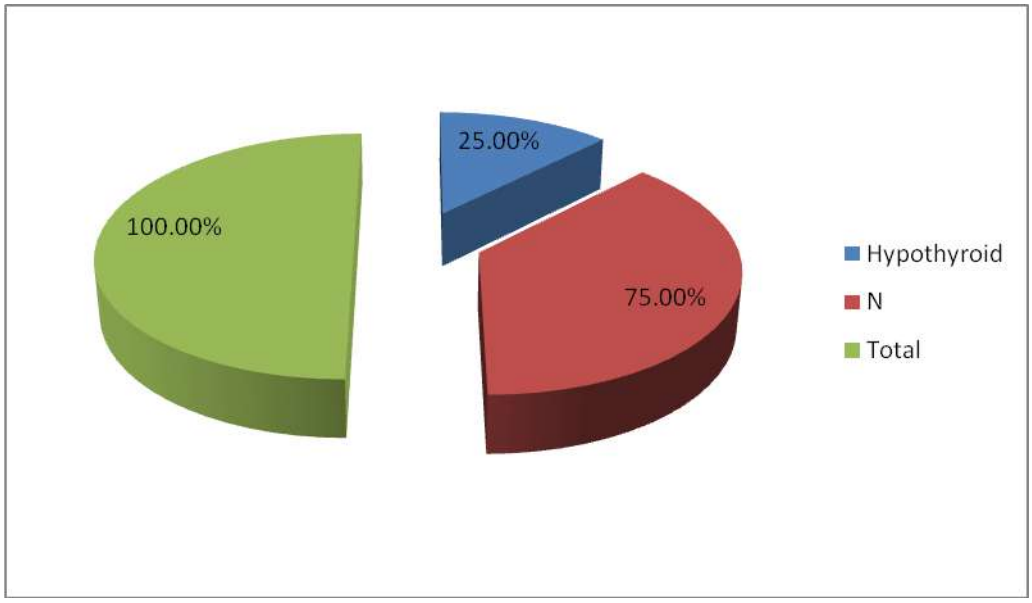

Figure 5: Co-morbidity

None of the patients enrolled had a positive family history of inflammatory bowel disease, neither any of the patients had undergone appendicectomy in past

None of the patients had any history of Alcohol, Smoking or Opium abuse, neither any of them had history of Intravenous Drug Abuse

Table 9: Previous treatment

	No. of cases	Percentage
IV Steroids	12	100.0%
Oral Steroids	11	91.7%
5-ASA	12	100.0%
AZA/6-MP	6	50.0%
AZA/6-MP (Intolerant)	2	16.7%
Biologics	4	33.3%
Cyclosporine	0	0.0%
Antibiotics	0	0.0%
Probiotics	0	0.0%

Looking at the past treatment history, all patients had exposure to 5-ASA compounds and received IV Steroids. Eleven (92%) patients had received oral steroids also. Six (50%) out of twelve patients were subjected to immunomodulators (azathioprine/6-mercaptopurine) previously, out of which four were naïve to immunomodulators and two were intolerant to immunomodulators. Four (33.3 %) out of twelve patients received biologics/biosimilars in the past whereas eight patients had not been on any biologics/biosimilars.

None of the patients received any Antibiotic, Probiotic or Cyclosporine/Tacrolimus in the past.

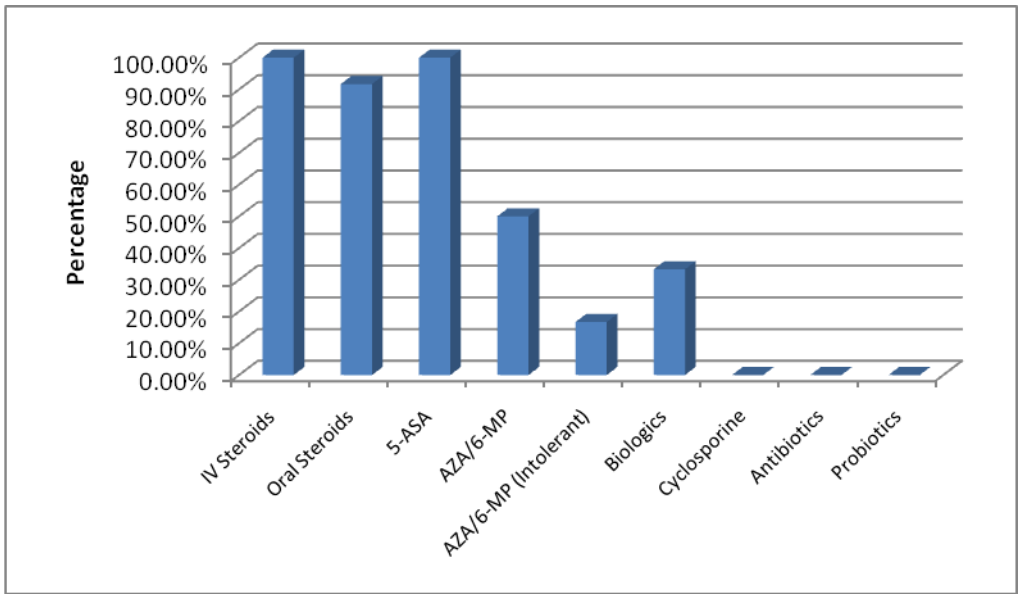

Figure 6: Previous treatment

Table 10: Retention Time

Retention Time	N	Minimum	Maximum	Mean	Std. Deviation
Baseline	12	0.2	4.0	2.5	1.2
Visit 2	12	0.8	5.0	3.4	1.3
Visit 3	12	2.0	6.0	3.9	1.2
Visit 4	12	3.0	5.0	3.9	0.7
Visit 5	12	2.5	6.0	4.5	0.9
Visit 6	12	3.0	6.0	4.5	1.1
Visit 7	12	1.0	5.0	4.1	1.1

The mean retention time (hours) was 2.5 ± 1.2 at baseline (First FMT). The average retention time increased at fourth FMT (3.9 ± 0.7) and further at seventh FMT (4.1 ± 1.1). Patients were instructed to retain the delivered slurry for as long as they can.

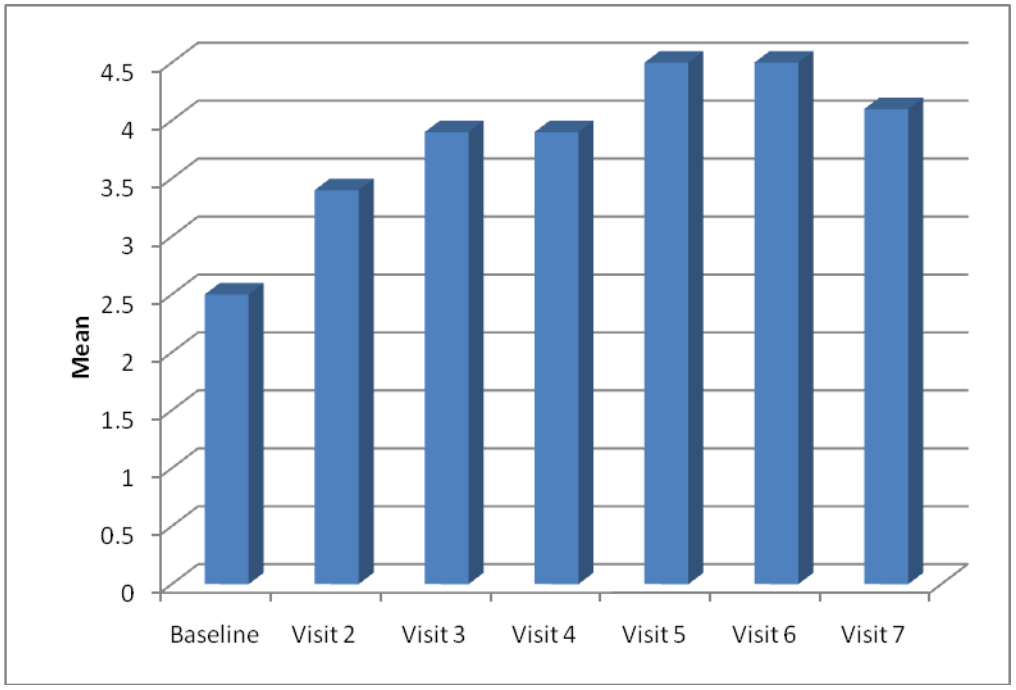

Figure 7: Retention Time

Table 11: hs CRP

	N	Minimum	Maximum	Mean	Std. Deviation
hs CRP	11	0.4	316.2	46.6	92.3

The mean hs CRP of patients included in the study was 46.6 ± 92.3

Table 12: Full Mayo Score

Full Mayo Score	N	Minimum	Maximum	Mean	Std. Deviation
Baseline	12	6.0	9.0	7.7	1.2
Visit 2	12	4.0	8.0	6.1	1.2
Visit 3	12	3.0	7.0	5.3	1.1
Visit 4	12	1.0	7.0	4.3	1.9
Visit 5	12	1.0	7.0	3.8	2.1
Visit 6	12	1.0	8.0	4.0	2.4
Visit 7	12	1.0	8.0	3.3	2.3

Mean Full Mayo Score was 7.7 ± 1.2 at baseline FMT which improved to 4.3 ± 1.9 and 3.3 ± 2.3at fourth FMT and seventh FMT respectively.

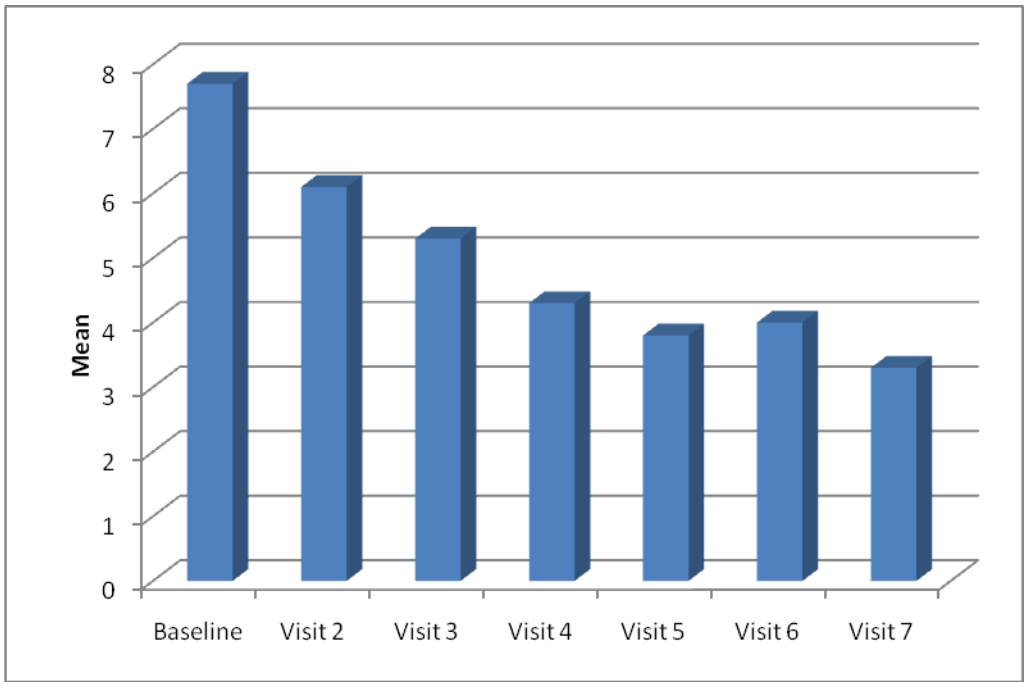

Figure 8: Full Mayo Score

Table 13: No. of stools

No. of stools	N	Minimum	Maximum	Mean	Std. Deviation
Baseline	12	1.0	3.0	2.4	0.7
Visit 2	12	1.0	3.0	1.7	0.8
Visit 3	12	0.0	2.0	1.4	0.7
Visit 4	12	0.0	2.0	1.2	0.8
Visit 5	12	0.0	2.0	1.0	0.9
Visit 6	12	0.0	3.0	1.3	0.9
Visit 7	12	0.0	3.0	0.8	0.8

Average number of stools as per Mayo Score was 2.4 ± 0.7 at baseline FMT. Number of stools decreased at fourth and seventh FMT's to 1.2 ± 0.8 and 0.8 ± 0.8 respectively.

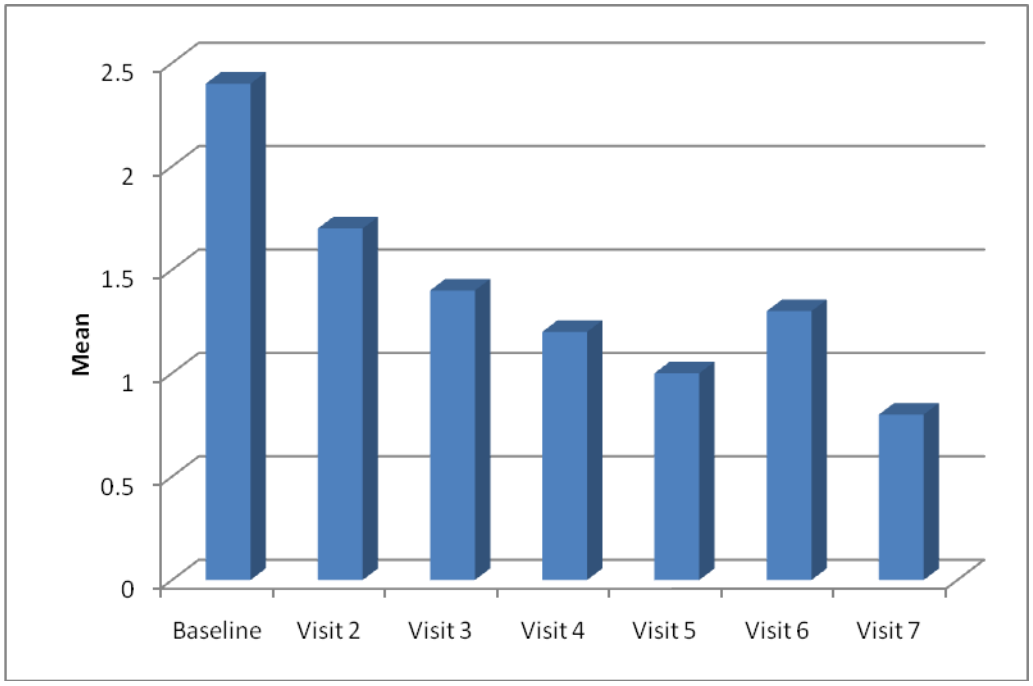

Figure 9: No. of stools

Table 14: Blood in stools

Blood in stools	N	Minimum	Maximum	Mean	Std. Deviation
Baseline	12	0.0	2.0	1.3	0.8
Visit 2	12	0.0	2.0	0.8	0.6
Visit 3	12	0.0	1.0	0.3	0.5
Visit 4	12	0.0	1.0	0.3	0.5
Visit 5	12	0.0	1.0	0.1	0.3
Visit 6	12	0.0	1.0	0.2	0.4
Visit 7	12	0.0	2.0	0.3	0.6

Blood in stools as per Mayo Score was 1.3 ± 0.8 at baseline FMT. Blood in stools decreased at fourth FMT to 0.3 ± 0.5 and remained almost the same (0.3 ± 0.6) at seventh FMT.

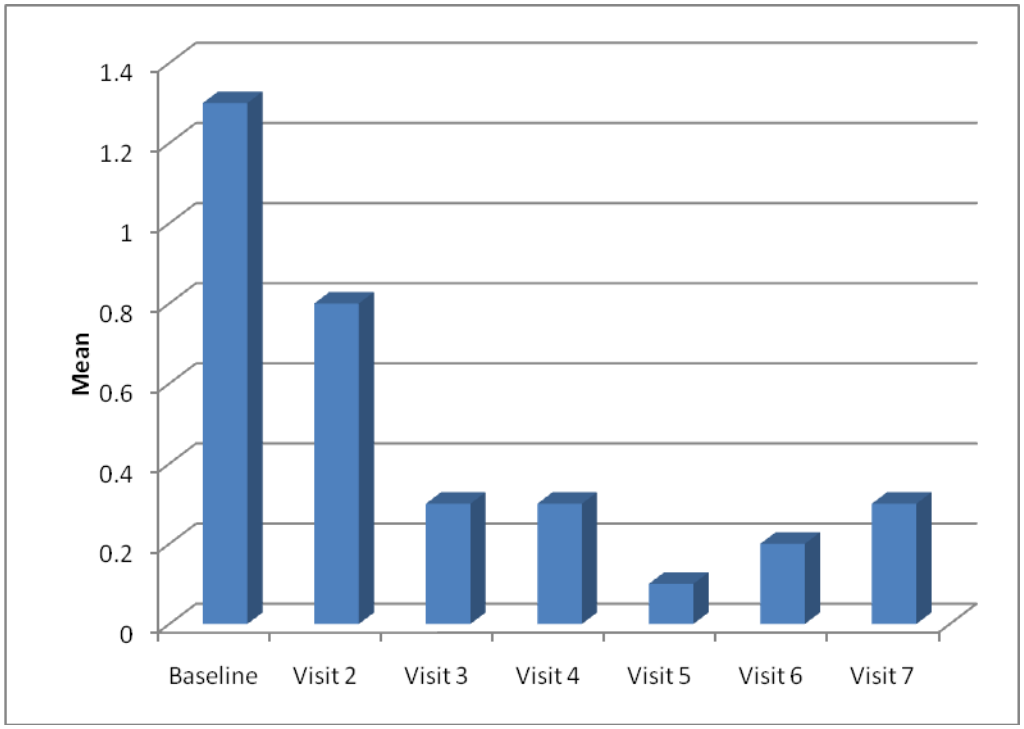

Figure 10: Blood in stools

Table 15: Endoscopic Mayo score

Endoscopic Mayo score	N	Minimum	Maximum	Mean	Std. Deviation
Baseline	12	2.0	2.0	2.0	0.0
Visit 2	12	2.0	2.0	2.0	0.0
Visit 3	12	2.0	2.0	2.0	0.0
Visit 4	12	0.0	2.0	1.5	0.7
Visit 5	12	0.0	2.0	1.3	0.9
Visit 6	12	0.0	2.0	1.2	0.9
Visit 7	12	0.0	2.0	1.0	1.0

Average Endoscopic Mayo Score was 2 ± 0 at baseline FMT which decreased to 1.5 ± 0.7 and further to 1.0 ± 1.0 at fourth and seventh FMT sessions respectively.

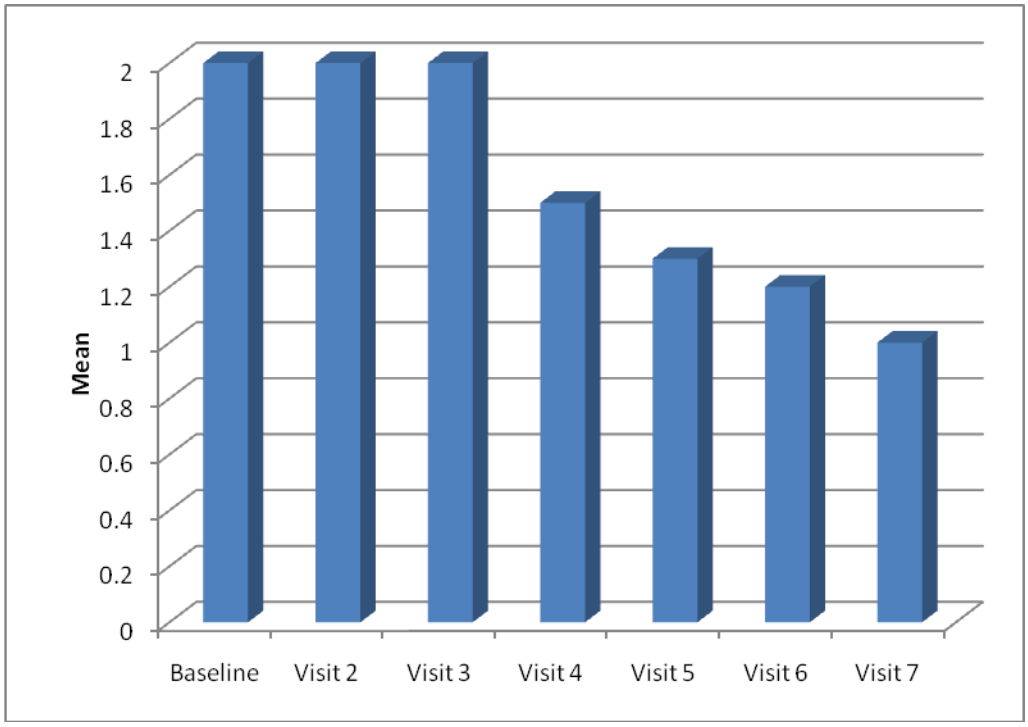

Figure 11: Endoscopic Mayo score

Table 16: Disease extent

Disease extent	**Baseline**		visit 2		visit 3		visit 4		visit 5		visit 6		visit 7	
0	No.	%age	No.	%age	No.	%age	No.	%age	No.	%age	No.	%age	No.	%age
E1	0	0.0%	0	0.0%	1	8.3%	1	8.3%	2	16.7%	2	16.7%	2	16.7%
E2	3	25.0%	3	25.0%	3	25.0%	3	25.0%	3	25.0%	3	25.0%	2	16.7%
E3	9	75.0%	9	75.0%	8	66.7%	7	58.3%	4	33.3%	3	25.0%	3	25.0%
Total	12	100%	12	100%	12	100%	11	92%	9	75%	8	67%	7	58%

Nine (75%) out of twelve patients had E3 disease, three (25%) patients had E2 disease and none of the patients had E1 disease at baseline FMT session. At fourth FMT session, seven (58.3%) out of twelve patients had E3 disease, three (25%) patients had E2 disease, one (8.3%) patient had E1 disease and one (8.3%) patient had achieved endoscopic remission. At seventh FMT session, three (25%) out of twelve patients had E3 disease, two (16.7%) patients had E2 disease, two (16.7%) patients had E1 disease and five (41.6%) patients were in endoscopic remission.

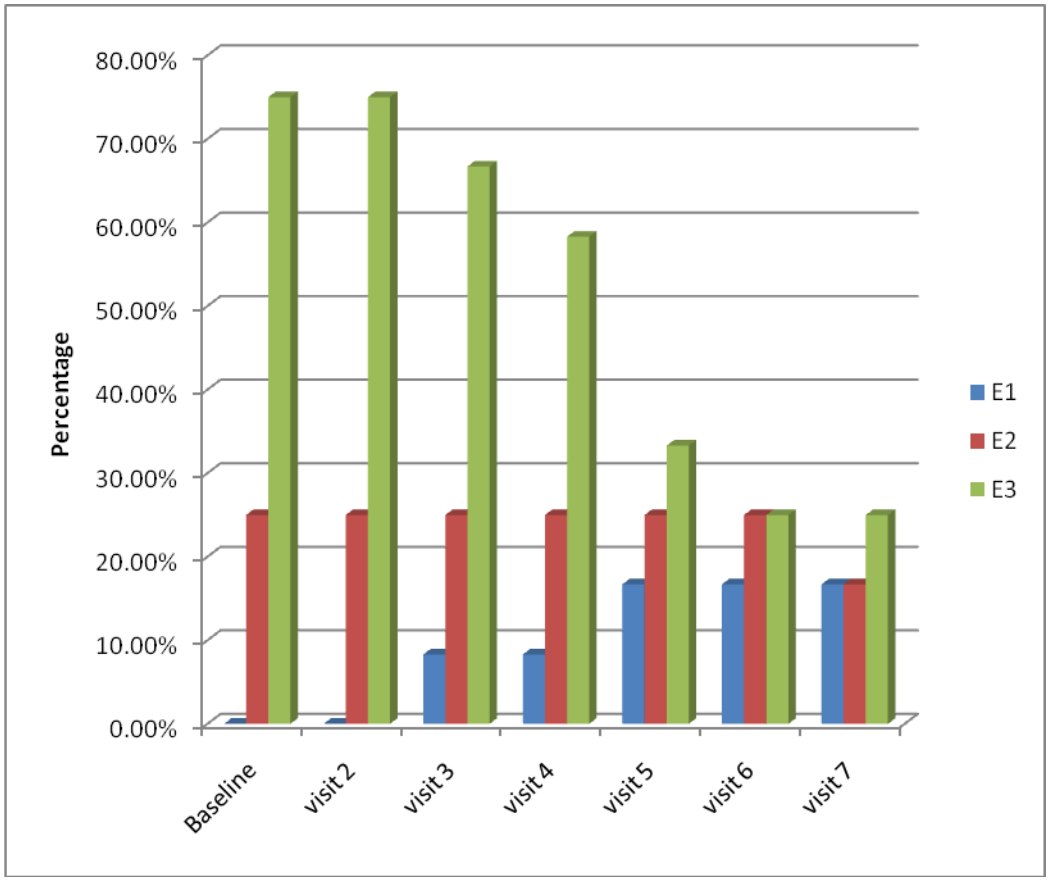

Figure 12: Disease extent

Table 17: Adverse events

	Baseline		visit 2		visit 3		visit 4		visit 5		visit 6		visit 7	
Adverse events	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
Fever	1	8.3%	1	8.3%	1	8.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Bloating	4	33.3%	1	8.3%	1	8.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Abdominal Distension	2	16.7%	1	8.3%	1	8.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Borborygmi	6	50.0%	2	16.7%	1	8.3%	1	8.3%	1	8.3%	1	8.3%	0	0.0%
Pain Abdomen	6	50.0%	2	16.7%	0	0.0%	1	8.3%	0	0.0%	0	0.0%	0	0.0%
Flatulence	1	8.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	8.3%	0	0.0%
Vomiting	2	16.7%	1	8.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Worsening of Diarrhoea	1	8.3%	3	25.0%	2	16.7%	1	8.3%	0	0.0%	0	0.0%	0	0.0%
Perianal Pain	0	0.0%	1	8.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

None of the patients had significant adverse event after FMT. Pain abdomen and borborygmi on the day of procedure (baseline FMT) were the most common adverse events noted followed by bloating. These were transient and improved over next 24-48 hours with symptomatic therapy. None of the patients had to be hospitalised for the adverse events. One patient had transient worsening of diarrhoea after baseline FMT which was managed conservatively. No evidence of transmission of Clostridium difficile was documented. As the number of FMT sessions, for a particular subject, increased; the tolerability of the procedure improved with fewer adverse events recorded.

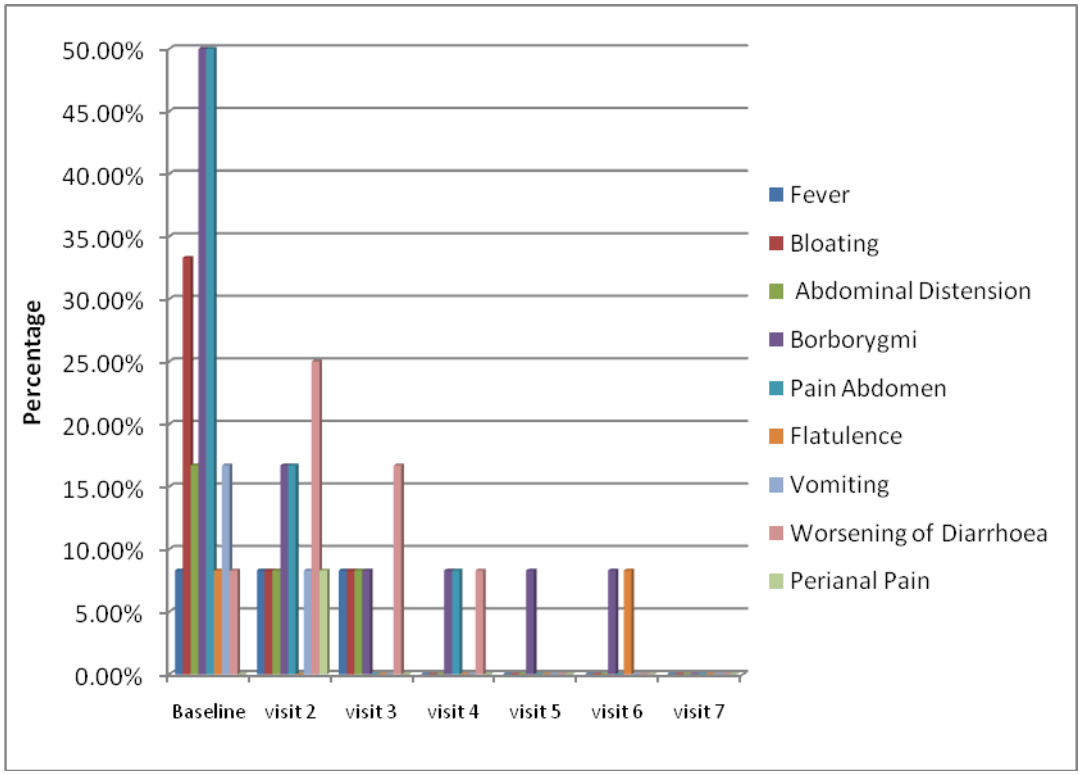

Figure 13: Adverse events

DISCUSSION

Faecal microbiota transplantation (FMT) targets gut microbiome dysbiosis and is emerging as a novel therapy for ulcerative colitis (UC). The efficacy of FMT in induction of remission in moderate-severe active UC has been addressed by four high quality randomized controlled trials (RCTs) [37-40] and multiple cohort studies. FMT has been shown to be effective in patients with steroid dependent UC [51,52] but studies in patients with steroid refractory UC are lacking except one trial from turkey.[41] Despite its unappealing nature, patients with active UC who are desirous for a cost effective, safe and efficacious therapy are open to considering this as a treatment, especially when recommended by a physician.

The results with FMT in UC have been conflicting.[37-40] The difference might be attributed to several reasons. First, composition of donor's microbiota may play a role as an important factor influencing the clinical improvement, as this treatment approach is donor dependent. In this study, we did not specify particular donors for particular subjects and used mixture of donors for each patient. Second, possible explanation for different results in clinical studies is delivery routes and number of FMT infusions. Third, the immune system of IBD patients is up regulated due to chronic inflammation, which might affect the therapeutic effect of FMT. The genetic build up of the recipient also defines the clinical response, apart from the different microbial composition of the donor faeces.

The role of FMT in active UC is still in a "transition zone" between experimental research and standard of care and further studies are needed to untangle the heterogeneity embedded in the previous randomized controlled trials.

This was an interventional, prospective and uncontrolled study. Patients enrolled in the study received FMT through per rectal route with colonoscope reaching up to ileum or as close as possible to caecum. None of the enrolled patients had a first or second degree relative with IBD, consistent with low prevalence of positive family

history of inflammatory bowel disease (IBD) in India as compared to west,[26] Addictions in the form of smoking/alcohol/opium/IVDU were not present in any of the patients. All of the patients received IV Steroids for disease control followed by oral steroids in eleven out of twelve patients. All the patients were on concomitant 5-ASA and six out of twelve patients received Azathioprine which is a standard practice for the treatment of UC in northern India. Out of twelve patients, four (33.3%) patients had received biologics/biosimilars in the past highlighting the severe course of their disease.

Patients were followed-up for their clinical response at each session of FMT and the disease activity was assessed by full Mayo Clinic Score. Faecal slurry retention time was noted for all patients. For first session of FMT, average retention time was 2.5 ± 1.17 hours, two out of twelve patients were able to retain the slurry for less than one hour. The proportion of patients retaining slurry for ≥ 4 hours gradually increased with the number of FMT sessions and eleven (91.6%) out of twelve patients retained faecal slurry for ≥ 4 hours by seventh session. Disease activity improved with each session (mean Full Mayo score 7.66 ± 1.15 at baseline vs 3.3 ± 2.34 at session 7) as is noted in another study from our institute.[53]

Patients in our study were recruited while being on steroids and addition of FMT by a multi-session protocol resulted in achievement of primary outcome ie clinical remission in six (50%) out of twelve patients. Clinical response and mucosal healing (secondary outcome) was achieved in eight (66.6%) patients and seven (58.33%) patients respectively. We attribute better response rates in our study to the use of a multi-session protocol of FMT over 22 weeks.

In another study recruiting patients with moderately severe steroid dependent UC, steroid-free clinical remission was achieved in 19 out of 41 (46.3%) patients, whereas clinical response and endoscopic remission were achieved in 31 out of 41 (75.6%) and 26 out of 41 (63.4%) patients, respectively.[51]

Another similar study from Turkey included patients with steroid dependent and patients refractory to biologics and steroids.

Investigators found that after FMT, 21 (70%) of the 30 patients showed clinical response, and 13 (43.3%) of the 30 patients achieved clinical and endoscopic remission at week 12. Nine patients (30%) were accepted as a nonresponder at the end of the week 12.[41]

Instillation of the fecal slurry in the upper GI tract may not be effective as it has been hypothesized that gastric acid can destroy Bacteroides and Firmicutes.[54] The RCTs with a colonic or rectal instillation of fecal slurry [39,40] have shown better response compared to the oral route of administration.[38]

As inflammation in UC starts in rectum and proceeds proximally, and dysbiosis is expected to be more in inflamed areas than the non-inflamed ones, so Colonoscopic route of instillation was chosen in this study. Unlike administration into upper GI tract, there was also no risk of aspiration in colonoscopic route.[55]

Patient preparation is important to ensure delivery of the fecal slurry to the desired segments (terminal ileum/caecum). Bowel lavage using PEG based solution was done one day prior to the procedure, as it may impact successful colonization of the donor microbiota. This also enabled mucosal assessment at each visit.

FMT was combined with standard of care IBD therapy. This approach allowed these therapies to work synergistically. Remodelling the intestinal microbiota by FMT in all likelihood also improved the patients' response to other therapies.

The procedure was relatively safe with no serious adverse events. Pain abdomen and borborygmi on the day of procedure (baseline FMT) were the most common adverse events noted followed by bloating. None of the patients had to be hospitalised for the adverse events. One patient had transient worsening of diarrhoea at baseline FMT which improved with conservative treatment.

Our study has a few limitations. First, there was no control arm to compare the efficacy of FMT. Second, we could not analyze the gut microbiome of the patients and donors due to financial limitations

SUMMARY AND CONCLUSION

Fecal microbial transplantation (FMT) provides to replace beneficial bacteria with more favorable microbiomes in recipient with dysbiosis and is an emerging novel therapy for Ulcerative Colitis.

This was an interventional, prospective and uncontrolled study in patients with steroid refractory UC. Twelve patients received FMT for a total of seven sessions per patient through per rectal route with colonoscope reaching up to ileum or as close as possible to caecum. Patients were followed-up for their clinical response at each FMT session and the disease activity was assessed by full Mayo Clinic Score.

Primary outcome ie clinical remission was achieved in six (50%) out of twelve patients. Secondary outcome ie clinical response and mucosal healing was achieved in eight (66.6%) patients and seven (58.33%) patients respectively.

The proportion of patients retaining slurry for ≥ 4 hours gradually increased with the number of FMT sessions and disease activity improved with each session (mean Full Mayo score 7.66 ± 1.15 at baseline vs 3.3 ± 2.34 at session 7)

The procedure was relatively safe with no serious adverse events. Pain abdomen and borborygmi on the day of procedure (baseline FMT) were the most common adverse events noted followed by bloating. None of the patients had to be hospitalised for the adverse events. The tolerability of the procedure improved with every progressive session of FMT with fewer adverse events.

Intensive-dosing, multidonor, faecal microbiota transplantation induces clinical remission and endoscopic improvement in active ulcerative colitis. However, procedure for FMT needs standardisation in terms of donor selection, dosing and stool preparation.

To conclude, in a resource limited country like ours, treatment with biologics could pose a financial burden and surgery could be of significant risk. FMT could be considered as a potential rescue treatment modality in patients with refractory UC.

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S. No.	Name	Age	Sex	Year of Diagnosis	Family History	Appendicectomy	Alcohol	Smoking	Opium	IVDU	Co-morbidity	BMI (kg/m2)	Previous Treatment								Retention Time (Hours)							hs CRP	Full Mayo Score							Number of Stools (Mayo Score)							Blood in Stools (Mayo Score)						
													IV Steroids	Oral Steroids	5-ASA	AZA/6-MP	Biologics	Cyclosporine	Antibiotics	Probiotics	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Baseline	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
1	R	18	M	2016	N	N	N	N	N	N	N	22.18	Y	Y	Y	Y	Y	N	N	N	3	3.5	3	3.5	2.5	3	4	8.3	8	8	6	5	4	2	2	3	3	2	2	1	1	1	2	2	1	0	0	0	0
2	B	40	F	2016	N	N	N	N	N	N	N	22.2	Y	Y	Y	N	N	N	N	N	3	3.5	3.5	4	4	4	4	21.2	8	6	6	3	3	3	2	3	2	2	1	1	1	1	2	1	1	0	0	0	0
3	S	40	M	2006	N	N	N	N	N	N	Hypothyroid	22.71	Y	Y	Y	Intolerant	Y	N	N	N	3.5	4	6	3	5	4	4	316.2	7	6	5	7	6	6	4	2	2	1	2	2	2	1	1	1	0	1	0	0	0
4	R	43	M	2005	N	N	N	N	N	N	N	29.8	Y	Y	Y	N	N	N	N	N	0.2	2	3	4	4	3	4.3	NA	7	6	6	6	6	6	5	3	2	2	2	2	2	1	0	0	0	0	0	0	0
5	M	25	M	2016	N	N	N	N	N	N	N	22.7	Y	Y	Y	Y	Y	N	N	N	3.5	3	2	4.15	4	4	5	40.9	6	6	7	6	6	5	4	1	1	2	1	2	1	1	1	1	1	1	0	0	0
6	G	39	F	2012	N	N	N	N	N	N	N	22.01	Y	Y	Y	Y	N	N	N	N	2.3	4.3	5	3	5	6	4	30.6	9	8	6	3	2	2	1	3	3	2	1	1	1	0	2	1	0	0	0	0	0
7	K	33	F	2018	N	N	N	N	N	N	N	21.35	Y	Y	Y	Y	Y	N	N	N	0.5	0.75	3	3	5	5	4	76.1	9	7	6	5	1	1	1	3	2	2	2	0	0	0	2	1	0	0	0	0	0
8	J	29	F	2014	N	N	N	N	N	N	N	22.5	Y	N	Y	Y	N	N	N	N	3	4	4	4	4	4	5	6.2	6	5	5	3	5	8	8	2	1	1	1	1	3	3	0	0	0	0	0	1	1
9	R	47	F	2004	N	N	N	N	N	N	N	24.21	Y	Y	Y	Y	N	N	N	N	2	5	5	4	6	4	4	0.4	7	6	4	3	3	4	2	2	1	1	0	0	1	0	1	1	0	0	0	0	0
10	D	18	M	2018	N	N	N	N	N	N	N	16.13	Y	Y	y	N	N	N	N	N	2	5	5	4	5	5	1	6.4	9	5	5	7	7	7	7	3	1	1	2	2	2	1	2	0	0	1	1	1	2
11	S	42	F	2017	N	N	N	N	N	N	Hypothyroid	22.2	Y	Y	Y	Intolerant	N	N	N	N	4	4	3.3	5	5	6	5	2.2	7	4	4	3	2	3	3	2	1	1	0	0	1	1	1	0	0	0	0	0	0
12	S	30	F	2017	N	N	N	N	N	N	Hypothyroid	20.54	Y	Y	Y	N	N	N	N	N	3	2	4	5	5	6	5	4	9	6	3	1	1	1	1	2	1	0	0	0	0	0	2	1	0	0	0	0	0

S. No.	Endoscopic Mayo Score							Physician Global Status							Disease Extent							Adverse Events							Concomitant Medications			
	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Baseline	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Oral Mesalamine (g)	Rectal Mesalamine (g)	Corticosteroids (mg)	AZA/6-MP (mg)
1	2	2	2	2	2	0	0	1	1	1	1	1	1	1	E3	E3	E3	E3	E3	0	0	Pain Abdomen, Bloating	Worsening of Diarrhoea, Abdominal Distension	Worsening of Diarrhoea	N	N	N	N	2.4	1	N	50
2	2	2	2	1	1	1	0	1	1	1	1	1	1	1	E3	E3	E2	E2	E2	E2	0	Pain Abdomen, Abdominal Distension	N	N	N	N	N	N	3.6	1	N	N
3	2	2	2	2	2	2	2	2	1	2	2	2	2	1	E3	E3	E3	E3	E1	E1	E1	Pain Abdomen, Bloating, Borborygmi	Worsening of Diarrhoea, Perianal Pain	N	N	N	N	N	4.8	N	N	Intolerant
4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	E3	E3	E3	E3	E3	E3	E3	Flatulence	N	Bloating	N	Borborygmi	N	N	4.8	N	N	50
5	2	2	2	2	2	2	2	2	2	2	2	2	2	1	E3	E3	E3	E3	E3	E3	E3	Bloating, Borborygmi	Bloating	N	N	N	N	N	4.8	1	N	100
6	2	2	2	1	0	0	0	2	2	2	1	1	1	1	E3	E3	E3	E3	0	0	0	Borborygmi	N	N	N	N	Borborygmi, Flatulence	N	3.6	1	N	N
7	2	2	2	1	0	0	0	2	2	2	2	1	1	1	E3	E3	E3	E3	0	0	0	Borborygmi	Borborygmi	Fever, Borborygmi	Pain Abdomen, Borborygmi	N	N		4.8	1	N	50
8	2	2	2	1	2	2	2	2	2	2	1	2	2	2	E2	E2	E2	E2	E2	E2	E2	Pain Abdomen, Vomiting	Pain Abdomen	N	N	N	N	N	4.8	0.5	N	100
9	2	2	2	2	2	2	1	2	2	1	1	1	1	1	E2	E2	E2	E2	E2	E2	E2	Pain Abdomen, Worsening of Diarrhoea, Vomiting	Pain Abdomen, Worsening of Diarrhoea, Vomiting	Worsening of Diarrhoea, Abdominal Distension	Worsening of Diarrhoea	N	N	N	4.8	0.5	N	100
10	2	2	2	2	2	2	2	2	2	2	2	2	2	2	E3	E3	E3	E3	E3	E3	E3	Fever, Borborygmi	Fever	N	N	N	N	N	3.6	0.5	N	N
11	2	2	2	2	1	1	1	2	1	1	1	1	1	1	E2	E2	E1	E1	E1	E1	E1	Pain Abdomen	N	N	N	N	N	N	3.6	0.5	N	Intolerant
12	2	2	2	0	0	0	0	3	2	1	1	1	1	1	E3	E3	E3	0	0	0	0	Borborygmi, Bloating, Abdominal Distension	Borborygmi, Bloating	N	N	N	N	N	3.6	N	N	N

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The Principal,
DMC, Ludhiana.

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 31.08.2018 has approved the plan of thesis of Pulkit Dhiman, a student of your college for DM Medical Gastroenterology along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Role of fecal microbiota transplantation in patients with steroid refractory ulcerative colitis

Supervisor
Dr. Ajit Sood
Prof. & Head,
Dept. of
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Co-Supervisor
Dr. Sandeep Singh Sidhu
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The student has to submit the four copies of thesis, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :
1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTORATE OF MEDICINE

In

GASTROENTEROLOGY

OF

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

FEBRUARY, 2018

DR. PULKIT DHIMAN

DEPARTMENT OF GASTROENTEROLOGY
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS
For the degree of	D.M. (Gastroenterology)
Name of the candidate	Dr. Pulkit Dhiman
Supervisor	Dr. Ajit Sood, Professor & Head, Department of Gastroenterology, DMCH
Co-supervisor	Dr. Sandeep Singh Sidhu, Professor, Department of Gastroenterology, DMCH
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INTRODUCTION: Faecal microbiota transplantation (FMT) as a potential treatment for inflammatory bowel disease (IBD) is an area of active current research, taking into account of its success in the treatment of Clostridium difficile-associated colitis. Faecal microbiota transplantation (FMT) is the artificial replacement of a 'dysbiotic' microbiota with one from a healthy individual. There is paucity of data for the role of FMT in patients with steroid refractory Ulcerative Colitis. This study aims to study the role of fecal microbiota transplantation in steroid refractory UC. FMT will be done through retention enemas or colonoscopy and patients will be looked for remission/response at week 22.

Aim of Study: To study the role of FMT in patients with steroid refractory Ulcerative Colitis.

Materials & methods: Interventional, prospective and uncontrolled study which will be conducted on patients attending the outpatient department or admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India.

Clinical Significance: FMT as a potential treatment option in patients with steroid refractory Ulcerative Colitis.

Keywords: FMT: Fecal Microbiota Transplantation, UC: Ulcerative Colitis

(Signature)	(Signature)	(Signature)	(Signature)
Candidate	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS"** Exist in the Department of Gastroenterology, Dayanand Medical College & Hospital and will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate himself/herself under our supervision and guidance.

Supervisor

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APPROVAL PROFORMA
BY
RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Pulkit Dhiman
Department	Gastroenterology
Topic of Thesis	ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS
Likely date of appearing in DM examination	May, 2020
Date of enrollment	30 August, 2018
Name of Head of Department	Dr. Ajit Sood
1) Supervisor **2) Co-Supervisor**	Dr. Ajit Sood Dr. Sandeep Singh Sidhu
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
1. HOD Pharmacology	1. HOD Physiology
2. HOD Surgery	2. HOD Pathology
3. HOD Pathology	3. HOD Pharmacology
4. HOD Medicine	4. HOD SPM
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Approved: Yes / No

Chairperson
Research Committee
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Research Committee

Approved: Yes / ~~No~~

Chairperson
Ethics Committee
Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	DR. PULKIT DHIMAN S/0 KAMALJIT SINGH 533/2, GURU NANAK STREET CHAURA KUAN, PATIALA, PUNJAB 147001
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE HOSPITAL, LUDHIANA
3	COURSE OF STUDY AND SUBJECT	DOCTORATE IN MEDICINE (D.M.) GASTROENTEROLOGY
4	DATE OF ADMISSION OF COURSE	30TH August, 2017
5	TITLE OF THE TOPIC	ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Inflammatory bowel disease (IBD) largely comprises Crohn's disease and ulcerative colitis which runs a relapsing, remitting course and tends to occur in the second or third decade of life.[1] The prevalence of IBD is greatest in the developed world affecting one million people in USA, 2.5 million in Europe and the prevalence is increasing in developing countries. Recent studies from China, India and Middle East reported prevalence of between 2.0 and 6.0 per 100,000 individuals.[2]

INFLAMMATORY BOWEL DISEASE (IBD) is the result of a chronic inflammatory process deriving from disequilibrium between self-microbiota composition and immune response. The etiology of IBD is still unknown, but several factors have been identified for induction and alteration of mucosal immune system and loss of immune tolerance, associated with dysbiosis (altered balance of beneficial and aggressive bacteria in gut).[3]

The human gastro-intestinal tract is home to about 1000 different species of bacteria along with viruses and yeasts which represents 100 fold greater genetic material than found in human body. It is involved in short-chain fatty acid metabolism, prevention of invasion by pathogens and preservation of gut epithelial barrier. Colonic microbiota is largely composed of members of two predominant phyla, gram positive Firmicutes (about 35%) and gram negative Bacteroides (about 60%). The remaining is representative of gram negative Proteobacteria, Actinobacteria and Eucharyotes.

Patients with IBD generally have lower bacterial diversity. Microbial segregation was found as compared to healthy individuals in terms of decreased Firmicutes and increase in population of Proteobacteria.[4]

Faecal microbiota transplantation (FMT) is the artificial replacement of a 'dysbiotic' microbiota with one from a healthy individual.

Current medical treatment of IBD remains imperfect and a significant number of patients need colectomy to manage their disease. FMT has emerged as a novel approach which targets the pathophysiology of IBD i.e. alteration in gut microbiome.

In recent years, many studies are coming up which report beneficial effect of FMT in induction of remission in patients with active Ulcerative Colitis. This study intends to look into the role of FMT in patients with steroid refractory Ulcerative Colitis.

6.2 REVIEW OF LITERATURE

It has long been thought that gut microbiota is likely to be the key to the pathogenesis of IBD. Early investigators considered Crohn's disease to be variant of Johne's disease, which is caused by Mycobacterium paratuberculosis and affects cattle.[5] It has been previously shown that infection with Campylobacter and non-typhoid Salmonella increases the risk of developing IBD.[6]

Recent genome wide association searches have uncovered over 150 potential genes associated with IBD.[7]

FMT have been used since fourth century when two Chinese physicians Ge Hong and Li Shizen first described the use of fresh or fermented faecal products, called 'yellow soup', to treat severe diarrhoea, fever, pain and constipation.[8]

FMT for the treatment of patients with pseudomembranous colitis was first reported in 1958.[9] In hindsight, these patients were in fact colonized by an overgrowth of toxigenic Clostridium difficile as is commonly seen in elderly patients treated with broad spectrum antibiotics. First report of the use of FMT to treat CDI came in 1983. The first randomized controlled trial for FMT in this setting was reported in 2013. Encouraged by the success of FMT in C. difficile, various trials have come up regarding the role of FMT in IBD.

In 1989, Justin Bennet and Mark Brinkman first reported sustained remission when Dr Bennet treated his own refractory UC with enema delivered stool from a disease free donor.[10]

In 2012 Angelberger et al. reported their results of FMT used to treat five patients with immunotherapy-refractory UC. Only one patient responded

following 3 days treatment using FMT by a combined nasojejunal plus enema-delivered approach and this patient's microbiota resembled that of the donor.[11]

A paediatric study with 10 patients aged between 7 and 21 evaluated the efficacy of freshly prepared faecal enemas for 5 days in treating mild to moderate UC. In this study, seven of the nine subjects showed clinical response within 1 week, six of the nine subjects maintained clinical response at 1 month with a significant reduction in the paediatric UC activity index.[12]

Moayyedi et al. reported the results of a double blinded randomized placebo controlled trial using FMT in the form of an enema administered weekly over 7 weeks to patients with active UC. In this study, clinical remission is seen in 9 out of 38 (24%) patients in the active arm compared to 2 out of 37 (5%) in the placebo group. Eight of the nine patients in remission remained in remission after one year. Patients who were in remission had an increase in microbial diversity and there were no differences in serious adverse events between the FMT and placebo. Investigators noticed that patients diagnosed within a year of treatment were more likely to respond.[13]

Rossen et al. undertook a double blinded RCT using the naso-jejunal route to deliver FMT to patients with mild to moderately active UC. They chose to use patient's own stool as placebo. At 8 weeks, they found remission in 6 of 23 (26%) donor-FMT treated vs. 8 of 25 (32%) autologous FMT treated patients which showed autologous FMT may not be entirely without biological activity.[14]

In a recent 3-centre study from Australia, the investigators suggested that treatment of UC is likely to require an intensive treatment course. They used

multiple donors (3–7 per treatment) in their intensive dosing placebo controlled RCT in patients with mild-moderate ulcerative colitis. Patients on steroids at entry had to undergo a mandatory taper over 8 weeks. The trial achieved its primary endpoint with 11 out of 41 (27%) patients in the active treatment arm compared to 3 out of 40 (8%) achieving steroid-free remission at Week 8 ($P = 0.021$) but endoscopic remission did not differ between the groups.[15]

In another study from Australia, investigators took a novel approach to FMT preparation. Donor and autologous stool were prepared in entirely anaerobic conditions. They showed that 12/38 (32%) patients achieved steroid-free remission in the active arm compared to 3/35 (9%) patients who received autologous FMT ($P = 0.02$) in the intention to treat (ITT) analysis. Similarly clinical response and clinical remission rates were 55% vs. 20% ($P < 0.01$) and 50% vs. 17% ($P < 0.01$), respectively.[16]

In a study from Turkey, researchers studied the efficacy of FMT by assessing the clinical and endoscopic response in patients with ulcerative colitis (UC) who had failed anti-inflammatory and immunosuppressive therapy.

They reported that twenty one of the (70%) thirty patients showed clinical response, and thirteen of the thirty (43.3%) patients achieved clinical and endoscopic remission at week 12. Nine patients (30%) were accepted as a non-responder at the end of week 12. No adverse events were observed in the majority of patients during FMT and 12 weeks follow-up.[17]

6.3 AIMS AND OBJECTIVES OF THE STUDY

To study the role of fecal microbiota transplantation in patients with steroid refractory Ulcerative Colitis.

7. MATERIALS AND METHODS

STUDY DESIGN

This interventional, prospective and uncontrolled study will be conducted on the patients attending the outpatient department or admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India. Patients coming to outpatient department or admitted indoor shall be offered to undergo FMT. Patients providing informed consent will be enrolled for the study. Patients will be educated about fecal microbiota transplantation (FMT) through a Patient Information Sheet (PIS) which will be provided to each patient. Donor faeces (200-300 gm) will be diluted in 200-300 ml normal saline making slurry. It will be filtered and particulate matter will be removed. The resultant solution will be administered to the patient through colonoscopy within 6 hours of preparation.

Patients will be encouraged to retain the solution for as long as possible. These FMTs will be done at week 0, 2, 6, 10, 14, 18 and 22 weeks. Steroids will be tapered (5 mg/week) after the enrollment of patients in FMT protocol.

7.1 SOURCE OF DATA

Patients suffering from steroid refractory Ulcerative Colitis and attending the outpatient department or admitted indoors in Department of Gastroenterology, DMC & H will be enrolled for the study.

7.2 METHOD OF COLLECTION OF DATA

Confirmed cases of Ulcerative Colitis who are refractory to steroids will be enrolled for the study and FMT will be done at week 0, 2, 6, 10, 14, 18 and 22 weeks through colonoscopy/retention enemas.

INCLUSION CRITERIA FOR PATIENTS

- Age ≥18 years.
- Patients who have moderately active disease based on Mayo Clinic score of 6 to 10 and are refractory to steroids (defined as ulcerative colitis that lack a meaningful clinical response to glucocorticoids up to doses of prednisone 40 to 60 mg/day or equivalent within 30 days for oral therapy or 7-10 days for IV therapy).
- Able to give informed consent and/or assent as appropriate.

EXCLUSION CRITERIA FOR PATIENTS

- Patients with severe disease (defined as Mayo Score of >10).
- Patients having toxic megacolon.
- Pregnant and lactating ladies.
- Prior colectomy.
- Positive stool test for any of the following: Clostridium difficile, Salmonella, Shigella, Yersinia, Campylobacter, E. coli by standard stool culture.
- Regular probiotic and antibiotic use within prior 4 weeks to enrollment
- Severe immunodeficiency, inherited or acquired (e.g. HIV, chemotherapy, or radiation therapy).
- Patients unwilling or unable to give consent or participate in all study requirements.

INCLUSION CRITERIA FOR DONOR

- Age >18 years.
- Score of 2,3 or 4 on Bristol stool scale.
- Stool culture and microscopy tests negative for common detectable enteric pathogens (Salmonella, Shigella, Campylobacter, Vibrio parahemolyticus, Vibrio cholera, Candida albicans, E. coli, Clostridium difficile, Entamoeba histolytica, Giardia lamblia, Cryptosporidium, Ascaris lumbricoides, Trematodes/tape worms).
- Negative screening for hepatitis A, anti HCV, HBsAg, syphilis and anti HIV within three months of donation.

EXCLUSION CRITERIA FOR DONOR

- Tattoos or body piercings within the last 6 months.
- High-risk sexual behaviors.
- Communicable illnesses or antibiotic treatment within the past 3 months.
- Metabolic syndrome, obesity (BMI of >30), or moderate-to-severe undernutrition (malnutrition).
- Intrinsic gastrointestinal illnesses such as irritable bowel syndrome, inflammatory bowel disease, gastrointestinal malignancies or major gastrointestinal surgical procedures.
- History of malignant illnesses or ongoing oncologic therapy.
- Ongoing immune-modulator therapy for any concurrent illness.
- History of chronic pain syndromes (fibromyalgia, chronic fatigue) or neurologic, neurodevelopmental disorders.

PROTOCOL FOR PERFORMING FMT

Donor preparation

- A gentle osmotic laxative will be given the night before procedure.
- Donor will be required to avoid any food to which the recipient may be allergic for 5 days before the procedure.
- Donor will be instructed to notify if any symptoms of infection occur between screening and time of donation.

Recipient preparation

- Large volume bowel preparation a day before the procedure.

PREPARATION OF STOOL AND ADMINISTRATION

- Stool sample will be collected in an airtight container and will be used within 6 hours of collection.
- Universal precautions will be observed while handling the stool.
- Preservative free normal saline will be used to dilute the stool sample.
- Using a blender, stool will be homogenized and diluents added till it reaches a liquid consistency.
- Stool will be filtered to remove the particulate matter.
- The finished stool slurry will be administered via colonoscope.

DURATION OF STUDY

The study period will start from 1st March 2018 till 31st August 2019

OUTCOME

Primary outcome

- Remission (defined as Mayo score of ≤2 with all individual categories ≤1)

Secondary outcome

- Clinical response (defined as decrease of at least 3 points and at least 30% at week 22 versus baseline)
- Mucosal healing (defined as Mayo endoscopy subscore ≤1)

STATISTICAL ANALYSIS

Chi square test shall be used in the study.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

- Yes, the study requires both investigations and interventions.
- Pre treatment tests:
    - Complete blood counts, Erythrocyte Sedimentation Rate
    - CRP- Quantitative
    - Serum creatinine, electrolytes
    - Random blood sugar
    - Anti HCV antibody, HBs Antigen, Anti HIV antibody
    - Stool routine, Stool culture
    - Stool for Clostridium difficile toxin/GDH
    - Cytomegalovirus serology
    - Colonoscopy
    - Histology if available
- Intervention:

  Faecal microbiota solution prepared from donor faeces will be given via colonoscope to the patients after taking an informed consent.

7.4 HAS ETHICAL CLEARENCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes attached herewith.

8. LIST OF REFERENCES

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2. Ng SC. Epidemiology of inflammatory bowel disease: focus on Asia. Best Pract Res Clin Gastroenterol. 2014;28:363–72.
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4. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2016;65:330–9.
5. Naser SA, Sagramsingh SR, Naser AS, Thanigachalam S. Mycobacterium avium subspecies paratuberculosis causes Crohn's disease in some inflammatory bowel disease patients. World J Gastroenterol. 2014;20:7403–15.
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7. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474:307–17.
8. Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? Am J Gastroenterol. 2012;107:1755–6.

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11. Angelberger S, Reinisch W, Makristathis A, Lichtenberger C, Dejaco C, Papay P, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. Am J Gastroenterol. 2013;108:1620–30.
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17. Uygun A, Ozturk K, Demirci H, Oger C, Avci IY, Turker T, et al. Fecal microbiota transplantation is a rescue treatment modality for refractory ulcerative colitis. Medicine (Baltimore). 2017;96(16):e6479.

PROFORMA

S.no.

CR no. Admn. no Date

Name Age Sex

Address

Contact number

Month and Year of diagnosis

Duration of disease

Addictions

Smoking No/Occassional/Regular

Alcohol No/Occassional/Regular

Opium No/Occassional/Regular

IVDU Yes/No

Appendicectomy: Yes/No

Comorbidity

HTN/T2DM/BA/CAD/COAD/Thyroid

disorder/CVA/Malignancy/Psychiatric illness/ Seizures/ Others

Family history of IBD: Yes/No (If Yes, specify)

BMI

Signature of Candidate **Signature of Supervisor**

Previous treatments:

	Dose	Duration
IV steroids		
Oral Steroids		
5-ASA		
AZA/6MP		
Infliximab		
Adalimumab		
Methotrexate		
Cyclosporine		
Tacrolimus		
Antibiotics		
Salazopyrin		
Budesonide		
Probiotics		
Others		

Signature of Candidate **Signature of Supervisor**

Mode of FMT:

Details of visits:

	Wk 0	Wk 2	Wk 6	Wk 10	Wk 14	Wk 18	Wk 22
DATE							
Stool quality (Bristol scale)							
Time of collection of sample							
Time of FMT							
Retention time							

	Wk 0	Wk 2	Wk 6	Wk 10	Wk 14	Wk 18	Wk 22
SYMPTOMS							
Number of stools (Mayo score) (A) 0=Normal 1=1-2/day 2=3-4/day 3=>4/day							
Blood in stools (Mayo Score) (B) 0 = None 1 = Visible blood with stool less than half the time 2 = Visible blood with stool half of the time or more 3 = Passing blood alone							
Nocturnal diarrhea							
Fever							
Pain abdomen							
Fatigue							
Weight loss							
Loss of appetite							
Joint pains							
Others							

Signature of Candidate **Signature of Supervisor**

INVESTIGATIONS (if available)							
TLC/Hb/Platelets							
ESR							
AST/ALT/ALP							
Albumin							
CRP-Quantitative							
Creatinine							
Electrolytes							
RBS							
HBsAg/Anti HCV/Anti HIV							
Stool R/E							
Stool C/S							
Stool C.diff toxin/GDH							
Stool atypical organisms							
Calprotectin (optional)							
CMV serology							
Others							

	Wk 0	Wk 2	Wk 6	Wk 10	Wk 14	Wk 18	Wk 22
DISEASE EXTENT							
Proctitis							
Proctosigmoiditis							
Left Sided Colitis							
Pancolitis							
Backwash ileitis							

	Wk 0	Wk 2	Wk 6	Wk 10	Wk 14	Wk 18	Wk 22
COLONOSCOPIC FINDINGS (MAYO SCORE) (C)							
0= Normal or inactive disease							
1= Mild disease (erythema, decreased vascular pattern, mild friability)							
2= Moderate disease (marked erythema, absent vascular pattern, friability, erosions)							
3= Severe disease (spontaneous bleeding, ulceration)							

Signature of Candidate **Signature of Supervisor**

ADVERSE EVENTS							
Fever							
Pain abdomen							
Nausea							
Vomiting							
Loss of appetite							
Worsening of diarrhea							
Abdominal distension							
Perianal pain							
Flatulence							
Borborygmi							
Bloating							
Rectal pain							
Constipation							
UTI							
URTI							
LRTI							
CONCOMITANT MEDICATIONS							
Drug 1/Dose							
Drug 2/Dose							
Drug 3/Dose							
PHYSICIAN GLOBAL ASSESSMENT (D)							
Normal (0) Mild (1) Moderate (2) Severe (3)							

	Wk 0	Wk 2	Wk 6	Wk 10	Wk 14	Wk 18	Wk 22
RESPONSE ASSESSMENT							
TOTAL MAYO SCORE (A+B+C+D)							

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o.....

am willing to participate in the study **"ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out of the study any time without assigning any reason.

.....
Signature of the Witness

Name:

.....
Signature of the patient

Signature of Candidate

Signature of Supervisor

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"ROLE OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH STEROID REFRACTORY ULCERATIVE COLITIS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ_____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ